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Award Number: DAMD17-01-1-0538

TITLE: Oncogenic Members of pp32 Family, the Widely Applicable
Targets for Immunotherapy in Human Breast Cancer

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REPORT DATE: June 2002

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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20030701 158

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

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|--|---|--|--|--|
| 1. AGENCY USE ONLY (Leave blank) | | 2. REPORT DATE June 2002 | 3. REPORT TYPE AND DATES COVERED Final (15 May 01 - 14 May 02) | |
| 4. TITLE AND SUBTITLE Oncogenic Members of pp32 Family, the Widely Applicable Targets for Immunotherapy in Human Breast Cancer | | | 5. FUNDING NUMBERS DAMD17-01-1-0538 | |
| 6. AUTHOR(S) Jining Bai, Ph.D. | | | | |
| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The Johns Hopkins University School of Medicine Baltimore, Maryland 21205 E-Mail: jnbai@jhmi.edu | | | 8. PERFORMING ORGANIZATION REPORT NUMBER | |
| 9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012 | | | 10. SPONSORING / MONITORING AGENCY REPORT NUMBER | |
| 11. SUPPLEMENTARY NOTES | | | | |
| 12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited | | | 12b. DISTRIBUTION CODE | |
| 13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information) This study is the initial phase of a feasibility study of a novel immunotherapeutic strategy for the treatment of breast cancer. The rationale is based upon recent findings that genes belonging to the pp32 family are differentially and alternatively expressed in most human breast cancers. In general, benign breast tissues express pp32, a tumor suppressor, whereas breast cancers express tumorigenic family members, including pp32r1 and pp32r2. Since pp32r1 and pp32r2 are expressed in nearly all breast cancers, but not in normal adult tissues, they may reasonably serve as targets for antigen-specific immunotherapy. The purpose of this study is to identify tumor-associated antigens (TAA) in pp32r1 and pp32r2, then test their suitability <i>in vitro</i> as immunotherapeutic targets in breast cancer. Currently, the second phase of (<i>in vivo</i>) feasibility is underway. If successful, the results may translate into eventual clinical trials of peptide vaccines or adoptive T cell therapy | | | | |
| 14. SUBJECT TERMS TAA, Immunotherapy | | | 15. NUMBER OF PAGES 12 | |
| | | | 16. PRICE CODE | |
| 17. SECURITY CLASSIFICATION OF REPORT Unclassified | 18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified | 19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified | 20. LIMITATION OF ABSTRACT Unlimited | |

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

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Introduction:

In this concept proposal, we proposed a feasibility study of a novel immunotherapeutic strategy for the treatment of breast cancer. The rationale is based upon recent findings that genes belonging to the pp32 family are differentially and alternatively expressed in most human breast cancers. In general, benign breast tissues express pp32, a tumor suppressor, whereas breast cancers express tumorigenic family members, including pp32r1 and pp32r2. Since pp32r1 and pp32r2 are expressed in nearly all breast cancers, but not in normal adult tissues, they may reasonably serve as targets for antigen-specific immunotherapy.

Body:

Since Statement of Work is not available for Concept Proposal, the list of Specific Aims will be used to report the progress of this project.

1) Specific Aim #1: Identify, synthesize and test candidate peptides that could potentially bind to HLA class I molecules based on the coding sequence of pp32r1 and pp32r2.

Using Bioinformatics and ImMunoGeneTics tools, we analyzed the entire coding region of pp32, pp32r1 and pp32r2 genes for binding affinity with HLA-A*0201 molecule as well as the degradation pattern by proteasomal cleavages. The result of calculation shown (Table 1) that 19 motifs are potentially favorable of binding to HLA-A*0201 molecule with high affinity. To verify the prediction *in vitro*, HLA-A*0201+ TAP-deficient T2 hybridoma (ATCC) was pulsed with 50ug/ml of each peptide representing the motif (or control) and 5ug/ml of b2-microglobulin for 18hr at 37 C. HLA-A*0201 expression was then measured by flow cytometry using mAb BB7.2 (ATCC) followed by incubation with FITC-conjugated secondary antibody. Fluorescent index of HLA-A*0201 to each peptide can be determined as: (mean fluorescence with peptide - mean fluorescence without peptide) / (mean fluorescence without peptide). The result shown 10 out of 19 motifs is capable of binding to HLA-A*0201 in a concentration dependent manner (Table 1).

2) Screen for candidate pp32r1 & pp32r2 peptides that fulfill the requirements for TAA.

In order to be qualified as a TAA, a motif has to be able to meet several criteria in addition to the binding to HLA-A*0201. These requirements include (i) the antigen can be naturally processed by tumor cells, (ii) it permits expansion of antigen-specific CTL; (iii) it is presented in a MHC-restricted fashion. CTL assay was carried out to test if the motifs identified in Aim#1 fulfill the requirements for TAA. In brief, Cr⁵¹-labeled target cells (T2 cells pulsed with peptide or cancer cell expressing pp32 family members) were incubated with various numbers of CTL effector cells for 4 hr. Cr⁵¹-release assays were performed in triplicate per condition using 5x10³ labeled target cells per well in a 96-well plate. Percent specific lysis will be calculated from CPM of (experimental result - spontaneous release)/(maximum release - spontaneous release). The results, summarized in Table 2, indicate that 2 out of 10 motifs fulfilled the above requirement as TAA.

3) Evaluate whether the pp32r1- or pp32r2- specific CTL can recognize a broad range of breast tumors (optional).

As an optional study proposed in the Concept Project, this study is currently under way in the PI's Lab to evaluate the widely applicability of the identified candidate TAA.

Key Research Accomplishments:

We have identified two peptide motifs from pp32 family members, which fulfill the requirement to be TAAs. This study (mostly *in vitro*) provided bases for further *in vivo* validation in breast cancer animal models.

Reportable Outcomes:

After the completion of the 3rd Specific Aim (Optional), we are expected to apply for U.S. Patent provision and to publish the results supported by this Concept Award.

Conclusions:

We demonstrated *in vitro* that (i) the oncogenic pp32 family members can be presented by HLA-A*0201, (ii) the HLA-A*0201 cells bearing these motifs can be recognized and lyzed by pp32r1- or pp32r2- specific CTL in a MHC class I specific manner.

| Peptide | BIMAS Score | LpRep Score | FPEITHI Score | Binding to T2 Cells |
|---------------|-------------|-------------|---------------|---------------------|
| 0801-01 | 3499.535 | 3.37 | 26 | + |
| 0801-02 | 1591.602 | 2.46 | 22 | - |
| 0801-03 | 805.719 | 5.76 | 17 | + |
| 0801-04 | 681.542 | 3.54 | 28 | + |
| 0801-05 | 636.316 | 4.19 | 25 | + |
| 0801-06 | 445.216 | 6.90 | 27 | - |
| 0801-07 | 481.542 | 3.13 | 26 | + |
| 0801-08 | 432.319 | 4.87 | 21 | - |
| 0801-09 | 399.682 | 7.69 | 23 | + |
| 0801-10 | 379.216 | 5.81 | 13 | - |
| 0801-11 | 301.331 | 3.12 | 27 | + |
| 0801-12 | 281.542 | 3.47 | 22 | - |
| 0801-13 | 264.498 | 6.72 | 24 | + |
| 0801-14 | 226.014 | 3.54 | 20 | - |
| 0801-15 | 212.775 | 6.43 | 19 | + |
| 0801-16 | 172.752 | 6.81 | 21 | + |
| 0801-17 | 148.896 | 5.87 | 24 | - |
| 0801-18 | 139.730 | 6.72 | 19 | - |
| 0801-19 | 105.719 | 7.99 | 18 | - |
| MGA1 (P.Ctrl) | 734.189 | 4.86 | 26 | + |
| ID9 (N.Ctrl) | 0.000 | n/a | 1 | - |

Table 1. Predicted HLA-A*0201 Binding Motifs and Their Ability to Bind T2 Cells.

| Peptide | CTL Lysis | Processing | CTL Expansion | MHC I Restriction |
|---------------|-----------|------------|---------------|-------------------|
| 0801-01 | + | n/a | Yes | n/a |
| 0801-03 | +++ | Yes | Yes | Yes |
| 0801-04 | + | n/a | Yes | n/a |
| 0801-05 | + | n/a | Yes | n/a |
| 0801-07 | +++ | Yes | Yes | Yes |
| 0801-09 | + | n/a | Yes | n/a |
| 0801-11 | - | n/a | No | n/a |
| 0801-13 | + | n/a | Yes | n/a |
| 0801-15 | - | n/a | No | n/a |
| 0801-16 | - | n/a | No | n/a |
| MGA1 (P.Ctrl) | +++ | Yes | Yes | Yes |
| ID9 (N.Ctrl) | - | No | No | No |

Table 2. Summary of CTL Assays for Motifs That are Capable of Binding to HLA-A*0201

Summary of Personnel Partially Supported by This Concept Award:

- 1) Jining Bai (PI)
- 2) Adetunke Jagun (Technician)

CURRICULUM VITAE

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Education & Training:

| | |
|-----------|--|
| 1983-1988 | B. Eng., Department of Engineering Physics, Tsinghua University, Beijing, P. R. China |
| 1990-1996 | Ph.D. / Graduate studies, Department of Biophysics, Johns Hopkins University Baltimore, MD |
| 1996-1999 | Post-doctoral Fellow, Division of Molecular Pathology Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, MD |

Professional Experiences:

| | |
|------------|--|
| 1985-1986 | Instructor, Computer programming School of Professional Studies Tsinghua University, Beijing, P. R. China |
| 1986-1988 | Research assistant, Institute of Material Sci. & Tech., Tsinghua University, Beijing, P. R. China |
| 1988-1990 | Graduate studies, Department of Biol. Sci. & Tech., Tsinghua University, Beijing, P. R. China |
| 1989-1990 | Teaching assistant, Biology Lab, Department of Biol. Sci. Tsinghua University, Beijing, P. R. China |
| 1991-1995 | Pre-doctoral Fellow, Department of Embryology Carnegie Institution of Washington, Baltimore, MD |
| 1992-1993 | Teaching Assistant, Reproductive Physiology Johns Hopkins University. |
| 1996-2000 | Research Fellow, Division of Molecular Pathology Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, MD |
| 2000-2001 | Research Associate, Division of Molecular Pathology Department of Pathology, Johns Hopkins Medical Institutions Baltimore, MD |
| 2001- 2002 | Instructor, Division of Molecular Pathology Department of Pathology, Johns Hopkins Medical Institutions Baltimore, MD |
| 2002- | Assistant Professor, Division of Molecular Pathology Department of Pathology, Johns Hopkins Medical Institutions Baltimore, MD |

Bibliography:

Refereed Publications

Bai J, Brody JR, Kadkol SS, Pasternack GR. Tumor suppression and potentiation by manipulation of pp32 expression. *Oncogene*, 20 (17):2153-60, 2001.

Bai, J., Kadkol, S. S., Brody, J. R. & Pasternack, G. R., pp32 gene family. *Encycl. Mol. Medicine*, Vol.4, 2564-2565, 2001.

Kadkol SS, El Naga GA, Brody JR, Bai J, Gusev Y, Dooley WC, Pasternack GR. Expression of pp32 gene family members in breast cancer. *Breast Cancer Res Treat.* 68(1):65-73, 2001.

Kadkol SS, Brody JR, Pevsner J, Bai J, and Pasternack GR Modulation of oncogenic potential by alternative gene use in human prostate cancer. *Nature Medicine* 5(3):275-279, 1999.

Bai J., Pagano RE. Measuring the spontaneous transfer and transbilayer movement of BODIPY-labeled lipids in liposome vesicles, *Biochemistry.* (36):8840-8848, 1997

Ding, J. R., Zhou, X., Bai, J., Liu, B. X. Amorphous niobium monoxide films prepared by reactive evaporation, *J. Vac. Sci. Tech.* 8:3349-51, 1990.

Absracts

Bai, J., Kadkol, S. S., Brody, J. R. & Pasternack, G. R. pp32 Gene family at the crossroad of oncogenesis and tumor suppression. *Proc. of NCC*, 2:151, 2000.

Bai, J., Kadkol, S. S., Brody, J. R. & Pasternack, G. R. alterations in pp32 gene family – A novel molecular targets in breast cancer therapy. *Proc. of 4th NMC*, SGK Foundation, 4:22, 2000.

Kadkol, S. S., Saria, E. A., Brody, J. R., Bai, J. & Pasternack, G. R.. Analysis of alternative pp32 gene use in breast cancer. *J. of Mol. Diagnostics* 1:58, 1999

Bai, J., Kadkol, S. S., Brody, J. R. & Pasternack, G. R. Modulation of Oncogenic Potential *in vitro* and *in vivo* by pp32. *Proc. AACR.* 90:574, 1999

Kadkol, S. S., Brody, J. R., Bai, J. & Pasternack, G. R. Heterogeneous expression of members of the closely-related pp32 gene family in prostate cancer and adjacent normal prostate. *Am. J. Pathol.* 153:1660, 1998

Bai, J. , Kadkol, S. S., Brody, J. R., Chamberlin, M., Cheong, R. & Pasternack, G.R. Cell- Type Specific Suppression of Proliferation of Human Prostatic Adenocarcinoma Cell by a Novel Tumor Suppressor pp32. *Proc. AACR.* Supl. 10:A-10, 1998

Invention & Patents:

Pasternack, G.R. & Bai, J. Method of Treating Cancer by Restoration of pp32 Function, *United States Patent & Trademark Office (USPTO)*, #60/118667, 1999.

Grants & Contracts:

Current:

- 1) National Research Award Komen Foundation Principal Investigator
Active (12/99-06/02) \$100,000 (annual direct)
Development of Novel Therapeutic Target and Approach for Breast Cancer
– Repairing Common Defects in Breast Cancer by Restoration of pp32.
- 2) IRG JHMI Principal Investigator
Active (05/01-05/03) \$20,000 (annual direct)
Development of a Novel Transgenic Mouse Model for Human Prostate Cancer
- 3) Idea Award DOD/CDMRP Principal Investigator
Active (10/01-10/04) \$100,000 (annual direct)
Identification of Widely applicable Tumor- Associated Antigens for Breast Cancer
ImmunoTherapy.
- 4) Pilot Award Breast Cancer SPORE/oncology Principal Investigator
Active (04/02-04/03) \$40,000 (annual direct)
HOXB7, Widely Applicable Targets for Immunotherapy against Breast Cancer.

Pending:

- 1) RO1 NIH Principal Investigator
Pending (01/03-12/06) \$225,000 (annual direct)
Localization and Molecular Interaction of pp32 Family Members

Complete:

- 1) Concept Award DOD/CDMRP Principal Investigator
Active (05/01-06/02) \$50,000 (annual direct)
Oncogenic Members of pp32 gene family, Widely Applicable Targets for
Immunotherapy against Breast Cancer.

Honors & Awards:

Honored Student, Tsinghua University (1983-1988)
Outstanding College Graduate Award, National Education Commission of China (1988)
Winner of Natural Philosophy Competition, Tsinghua University (1990)
Travel Award, European Symposium in Signal Transduction (1991)
Carnegie Fellowship, Carnegie Institution of Washington (1990-1991)
Dean's Fellowship, Johns Hopkins University (1990-1995)
Pathology Fellowship, Johns Hopkins Medical Institution (1996-1999)
National Research Award, Susan G. Komen Breast Cancer Foundation (1999-2001)
Concept Award, Congressionally Directed Medical Research (2000-2001)
Idea Award, Congressionally Directed Medical Research (2001-2004)

Invited Lectures:

- 1) Alterations in pp32 Gene Family – A Novel Molecular Targets in Breast Cancer Therapy.
The 4th National Mission Conference for Breast Cancer
Washington D.C.
September, 2000
- 2) pp32 Gene Family, Potential Therapeutic Targets for Breast Cancer and Prostate Cancer .
National Cancer Institute
Beijing, P.R. China
October, 2000
- 3) pp32 Gene Family at the Crossroad of Oncogenesis and Tumor Suppression.
The Cancer Congress 2000
Beijing, P.R.China, October, 2000